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THE BACTERIAL BLIGHT OF BEANS CAUSED BY BACTERIUM PHASEOLI[1]

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INTRODUCTION

Since the intensive culture of any crop results in the introduction and increase of infectious diseases, a general survey of the occurrence of bean maladies was undertaken in Wisconsin. From observations made it was evident that the principal diseases which occur in that State are the bacterial blights, caused by the widespread *Bacterium phaseoli* EFS. and *Bact. medicaginis* var. *phaseolicola* Burk. and the relatively unimportant *Bact. flaccumfaciens* Hedges. Anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. et Magn.) B. et C. and a fungous root rot caused by *Fusarium martii phaseoli* Burk. are of little consequence except in certain years when conditions are extremely favorable for their development. Mosaic is of common occurrence and often causes severe damage, particularly to the Refugee varieties.

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Because of its almost universal presence and the great loss it occasions, a study of *Bacterium phaseoli* was initiated. This organism and the disease it produces were described more than 30 years ago. It was not until recently, however, that bean blight was separated into three distinct bacterial diseases, as noted above. Since they now occur more or less intermingled and produce symptoms that overlap in their characteristics, it is probable that in the past they were all grouped with the common bean blight caused by *Bact. phaseoli*. Previous investigators in working with *Bact. phaseoli* have given chief attention to the bacteriological characters of the pathogene. It is the purpose of this bulletin to review briefly the known features of the disease and to give a detailed account of the writer's investigations dealing with the environmental factors that influence dissemination, infection, and varietal resistance, and especially with the relationship of the parasite to the host.

HISTORY OF THE DISEASE

The disease of beans caused by *Bacterium phaseoli* is probably of American origin. It was reported by Halsted (*15*),[3] who stated that the disease was first brought to his attention by a large seed company in New Jersey. Later, Smith (*32*) isolated, named, and described the organism. Delacroix (*9*) recorded a disease of beans near Paris that may have been the same as that described by Smith. A further account of the cultural characters of the organism was published in a second paper by Smith in 1901 (*33*). In the same year Halsted (*16*) described the field symptoms and experimented with possible preventive measures such as irrigation, shading, sprinkling, and crop rotation. Sackett (*30*) and Whetzel (*39*) each published a brief account of the disease. Fulton (*13*) described the field characteristics of the disease and discussed measures of control. He experimented with the hot-water seed treatment and spraying, and tested the comparative resistance and susceptibility of a number of varieties. Edgerton and Moreland (*11*) published an account of bacterial blight from the standpoint of the resistance of the organism to drying. They experimented with seed treatment and concluded that benetol and corrosive sublimate had given good results. They suggested also the use of home-grown seed.

That the organism can live over winter in diseased bean trash and may also be carried to clean fields in manure was shown by Muncie (*25*). His seed treatments with chemical solutions and with moist and dry heat failed to give results. He recommended the use of clean seed obtained by pod selection. In a general survey of the disease Rapp (*28*) reported that seed 2 to 3 years old produced blight-free plants. Seed treatments with chemicals and hot water he found to be impracticable.

The vascular nature of the disease was first reported by Barss (*2*), who found by internal microscopic examination that the xylem vessels of the affected plants were filled with bacteria not only toward the base but usually throughout the entire stem and even out into the branches, petioles, and veins. He likewise traced the bacteria

[3] Italic numbers in parentheses refer to Literature Cited, p. 34.

in many instances from the xylem vessels of the main stem to the suture of the pod. He showed that the bacteria entered through the vascular system of the seed without producing any outward symptoms. He stated that the death of seedlings can be accounted for by invasion into the vascular system from infected cotyledons.

Coincidently with Barss, Burkholder (*5*) likewise reported the blight of beans as a systemic disease and showed that the organism produced wilt symptoms by plugging the vessels. He also stated that since the disease is of a systemic nature, the seeds may become infected without producing lesions on the pods, a fact which is of considerable importance from the standpoint of control.

A comparison of the cause of soybean pustule, *Bacterium phaseoli sojense* Hedges, with *Bact. phaseoli* was made by Hedges (*18*), who worked principally with the cultural characteristics of both organisms.

Rands and Brotherton (*27*) experimented with several of the common bean diseases and made tests for disease resistance with 170 varieties of beans from the United States and 493 varieties obtained from 23 foreign countries.

A new bacterial disease of beans caused by *Bacterium flaccumfaciens* was reported by Hedges (*19*), who named and described the organism and made a thorough study of the cultural characteristics in comparison with *Bact. phaseoli.*

Burkholder (*7*) described a new bean disease caused by *Bacterium medicaginis* var. *phaseolicola* as being widespread in New York. He named and described the organism and gave the general characters of the disease in comparison with those caused by *Bact. phaseoli* and *Bact. flaccumfaciens.*

A report on the serological differentiation of *Bacterium campestre* EFS., *Bact. flaccumfaciens*, *Bact. phaseoli*, and *Bact. phaseoli sojense* was published by Link and Sharp (*22*). They showed that these four pathogenes could be differentiated by agglutination tests and that serologically *Bact. campestre*, although distinct from *Bact. phaseoli* and *Bact. phaseoli sojense*, was closely related to them and more remotely related to *Bact. flaccumfaciens.* Sharp (*31*) reported on morphological, physiological, and serological studies together with virulence and acid agglutination studies of *Bact. flaccumfaciens*, *Bact. phaseoli*, and *Bact. phaseoli sojense* and concluded that these three species all differ and can be differentiated by the use of the agglutination test. *Bact. flaccumfaciens* serologically stands apart from *Bact. phaseoli* and *Bact. phaseoli sojense*, which are very closely related.

HOST PLANTS

It is generally recognized that practically all the commercial varieties of the common bean, *Phaseolus vulgaris* L., are susceptible to the bacterial blight caused by *Bacterium phaseoli.* The studies of other workers have shown that the following are also hosts of the organism: The Scarlet Runner, *P. coccineus* L.; the civet bean, *P. lunatus* L.; the Lima bean, *P. lunatus* var. *macrocarpus* Benth.; the pinto bean, a variety of *P. vulgaris;* the white tepary bean, *P. acutifolius latifolius* Gray; the moth bean, *P. aconitifolius* Jacq.; the adzuki bean, *P. angularis* (Willd.) W. F. Wight; the urd bean,

P. mungo L.; and the black-eyed cowpea, *Vigna sinensis* (L.) Endl. Gardner (*14*) has reported that the trailing wild bean, *Strophostyles helvola* (L.) Britton, is a wild host. In the writer's inoculation work it was observed that the hyacinth-bean, *Dolichos lablab* L., is also susceptible.

DISTRIBUTION AND ECONOMIC IMPORTANCE

The geographical distribution of bacterial blight is not completely known. It probably occurs in every State in the Union, but little is known about its occurrence in other parts of the world. Delacroix (*9*) reported what appears to have been the same disease from France, and Ideta (*20*) reported it from Japan in 1903. It was also reported from the Philippine Islands by Reinking (*29*) in 1919, and from South Africa in the same year by Doidge (*10*). Skoric, formerly of the laboratory of plant pathology, University of Wisconsin, in conversation with the writer, stated that the same disease is widespread in Yugoslavia.

Next to anthracnose, bacterial blight is the most important disease of beans. According to the Plant Disease Reporter,[4] it was reported from New York in 1918 that the blight was prevalent in 75 per cent of the bean fields and caused very serious damage. In 1919 the disease caused an extremely high loss throughout the bean-growing districts. In that year Colorado suffered a loss of from 40 to 60 per cent of the crop, with a decrease in yield of about 35 per cent. In New York, which produced about 1,660,000 bushels of dry beans in 1918, or nearly one-eleventh of the total yield in the United States, it was estimated that from 5 to 10 per cent, with a possible average of 7 per cent, or about 125,000 bushels, was lost through the disease.

In 1921 this disease continued to be a very serious menace to the bean industry. It was prevalent over the entire eastern United States except in Vermont and New Hampshire. The most severe loss was in Michigan, estimated to be 25 per cent of the crop. In 1922 the total loss throughout the United States was about 10 per cent, and the same was true in 1923. In 1924 New York reported serious losses, even greater than in the previous year, and a reduction in yield estimated at 10 per cent. In 1925 the largest losses were reported from New York, Louisiana, Indiana, and Iowa. Reduction in yield averaged about 15 per cent. The damage in 1926, caused by the bacterial blight, was about the same as in the previous year, New York reporting the largest loss (10 to 15 per cent damage), while New Jersey, Ohio, Colorado, and Arizona all reported losses ranging from 5 per cent and lower.

In 1927 reports again showed serious losses from the blight, Indiana and Louisiana reporting losses of 4 per cent of the crop; Michigan and Montana, 3 per cent; Connecticut, Maryland, Virginia, Wisconsin, Minnesota, Mississippi, and Texas, 1 to 1.5 per cent. Linford, reporting from Utah, estimated a killing of from 5 to 95 per cent of the plants in numerous fields. The average loss through-

[4] UNITED STATES DEPARTMENT OF AGRICULTURE, BUREAU OF PLANT INDUSTRY. PLANT DISEASE SURVEY BULLETIN. v. 2, 1918; v. 3, 1919; v. 4, 1920; v. 5, 1921; v. 6, 1922; v. 7, 1923; v. 8, 1924; v. 9, 1925; v. 10, 1926; v. 11, 1927; v. 12, 1928. 1918-1928. [Mimeographed.]

out the United States was estimated at 1.4 per cent, or a total of approximately 226,000 bushels of beans.[5]

SYMPTOMS

Although the symptoms of bacterial blight have been accurately described by several investigators, there is included here a description of its appearance as observed on different parts of the bean plant at various stages of maturity.

Probably the most striking evidence of the disease is on the leaves. Here the lesions first appear on the lower side as small, water-soaked spots in the center of which, as they develop, a slight incrustation of bacterial exudate is found. The lesion is surrounded by a yellowish halolike zone. These lesions gradually enlarge and may coalesce with others, producing a brown scaldedlike area which in time causes a defoliation of the plant.

Bacteria from diseased seeds often produce very peculiar lesions on the first primary leaves. Here large angular water-soaked areas appear on the opposite leaves at similar positions, indicating that the initial infection took place while the leaves were still folded between the cotyledons. These differ from the small water-soaked circular secondary lesions in that they are larger and decidedly angular. On the young trifoliolate leaves a very unusual symptom appears in many cases when infection is severe, the bacteria entering the small veinlets, causing a slight discoloration of the adjacent tissues and a retardation of development in this region. With a rapid growth in the uninfected areas, a puckered appearance, very similar to mosaic symptoms, is produced.

When the bacteria are found in the vascular tissues of the leaf, another characteristic symptom can be seen. (Pl. 1, C.) The infection usually begins in the small veinlets, subsequently involving the larger veins and finally the midrib. In the case of severe infection the bacteria produce a reddish discoloration with a water-soaking of the tissues immediately surrounding the veins. When the leaf infection starts from the petiole, the main vein and its branches first appear water-soaked, later taking on a brick-red discoloration.

When diseased seeds are planted they may produce seedlings that exhibit a characteristic wilting in the case of severe infection. The first macroscopic appearance is a slight flagging or drooping of the leaves at the region of the pulvinus. (Pl. 1, B.) From isolation and microscopic observation of such tissues bacteria are generally found in large masses. During the night such leaves appear quite normal and turgid, but during the heat of the day they again become flaccid. In more advanced stages this drooping is followed by a wilting that in some cases may involve the entire seedling, whereas in others only a portion of the plant may be affected.

On the stem the pathogene may cause various types of lesions. The young seedling lesion begins as a small water-soaked spot which gradually enlarges, appearing somewhat similar to pod lesions. The necrotic areas are sometimes sunken and later appear as reddish dashes, extending longitudinally along the stem. (Pl. 1, E.)

[5] UNITED STATES DEPARTMENT OF AGRICULTURE, BUREAU OF PLANT INDUSTRY. Op. cit.

The surface of these spots is often split (pl. 1, B), and bacterial exudate can sometimes be seen oozing from the lesions (pl. 1, A).

When the plants are in the initial stages of pod formation, a lesion known as stem girdle or joint rot appears (pl. 1, D), which is first manifest as a small water-soaked spot either at the cotyledonary node or at other nodes along the stem. Upon enlargement, the lesion finally encircles the stem. The girdling is usually completed at the time when the pods are half mature, and the affected plant is so weakened by the increasing weight of the top that the stem breaks at the diseased node.

Bacterial blight causes much damage to the pods. The initial lesions first appear as minute water-soaked areas which gradually enlarge, accompanied by a discoloration and distinct zoning. Later the spot becomes dry and sunken and takes on a brick-red color. Often a yellowish-white incrustation of bacterial exudate can be seen covering the lesion. (Pl. 1, F.)

The bacteria may also infect the pod by way of the vascular elements. Following the dorsal suture, they cause a discoloration of this region and a water-soaking of the surrounding tissue. (Pl. 1, I.) In a like manner the organism may attack the seeds, producing several types of lesions. When infection occurs while the pods are young the seeds may rot entirely, or they may become so severely infected that only the shriveled seed coat remains. On the other hand, the bacteria entering by way of the funiculus may cause a discoloration at the hilum. (Pl. 1, G and H.) On dark-seeded varieties these discolorations are often difficult to detect, but on light-seeded varieties they are very noticeable. (Pl. 1, G.) On light-colored seeds when the infection is severe the lesions cover a considerable area and have a varnishlike appearance.

MOISTURE AS A FACTOR INFLUENCING INFECTION

It is generally agreed by various investigators that the chief external factors influencing stomatal movement are light and temperature. Some believe that humidity greatly affects the degree of stomatal openings, whereas others consider it of only minor importance. Wilson and Greenman (*40*) found that the stomata on plants of *Melilotus alba* L. that were left in a saturated atmosphere were well open, but the stomata of the plants that remained in the drier open air exposed to approximately the same light were nearly all closed. Darwin (*8*) showed that stomata were very sensitive to changes in humidity, closing when taken from a high to a low humidity and opening under the opposite conditions. Lloyd (*23*) believes that there is but little evidence to show that a high relative humidity favors the wide opening of the stomata in the ocotillo, and in *Mentha piperita* L., also a desert plant. Poole and McKay (*26*), on the other hand, believe that while light may be considered a fundamental factor in the movement of the stomata of the beet, yet stomatal closure is affected by low relative humidity even though the light is active.

Difficulty has often been experienced in obtaining stomatal penetration in the greenhouse with bacterial plant pathogenes when the inoculated plants were not placed under conditions of high humidity.

The necessity of such conditions for infection with *Bacterium phaseoli* has been known for some time. Smith (*35*) in working with this and other bacterial plant pathogenes always subjected the host plants used to a moisture treatment previous to inoculation, in order to be insured of good infection. With this in mind, inoculation experiments were performed by the writer to determine, if possible, the relationship of moisture to stomatal movement in bean leaves. The principal varieties used for this work were Wardwell Kidney Wax and Wells Red Kidney, both of which are very susceptible to the blight.

The cultures of *Bacterium phaseoli* used for inoculation were received from a number of sources. Two came from the United States Department of Agriculture, Washington, D. C.; one from J. H. Muncie, Michigan Agricultural Experiment Station; one was isolated by the writer from beans gathered at Racine, Wis.; and one was isolated from material collected at Madison, Wis. These cultures all gave similar results and will therefore not be considered separately in the following discussion. A culture of *Bact. medicaginis* var. *phaseolicola* isolated from beans collected at Columbus, Wis., was also used. The bacteria were allowed to grow on potato-dextrose agar slants (pH 7) for a period of about a week at a temperature of 28° C. They were then removed by washing them into sterile atomizers containing sterile distilled water, and this suspension was used as the inoculum.

Since the pathogenicity of *Bacterium phaseoli* has been demonstrated by other investigators, results on this phase of the problem will not be recorded here.

The plants for inoculation purposes were grown in 4-inch pots until they reached a height of about 8 inches. These seedlings were grouped into three lots. The first lot was covered completely with a glass container in order to produce a saturated atmosphere about the plant and was allowed to remain in place for a period of 24 hours, after which the plants were sprayed with a suspension of the bean-blight organism and again covered for 24 hours. The second series of plants was not given a premoist treatment as was the first lot, but was otherwise treated the same. The plants were covered after inoculation. The third set was inoculated without a premoist treatment or a covering after inoculation. All the plants in the three series were then placed under similar conditions in a greenhouse. The plants were examined daily for water-soaked lesions. Both of the organisms used in these tests, *Bacterium phaseoli* and *Bact. medicaginis* var. *phaseolicola*, produced numerous water-soaked lesions, and in each series platings were made in order to be positive that the lesions were being caused by the respective pathogenes used. The results of these experiments are given in Table 1.

TABLE 1.—*Degree of infection caused by Bacterium phaseoli and Bact. medicaginis var. phaseolicola on bean plants subjected to moisture treatments of various duration*

[The letters a, b, and c each represents a single inoculation experiment]

Treatment	Degree of infection	
	Bact. phaseoli	Bact. medicaginis var. phaseolicola
1. Covered for 24 hours, inoculated, and covered for 24 hours.	a, Heavy b, Heavy c, Severe	a, Heavy. b, Heavy. c, Heavy.
2. Inoculated and covered for 24 hours	a, Medium b, Heavy c, Heavy	Not performed.
3. Inoculated and not covered	a, Very little b, Very little c, Very little	a, Very little. b, Very little c, Very little.

Little difficulty was experienced in obtaining stomatal penetration with *Bacterium medicaginis* var. *phaseolicola*. Numerous water-soaked lesions were obtained in one case without placing the inoculated plants in a moist chamber; however, the greenhouse in which the plants were kept was maintained at a high temperature and humidity. When inoculations were made under controlled moisture conditions for 24 hours numerous infections were always in evidence.

Burkholder (*7*) was unable to obtain stomatal penetration, even though his plants were placed in a moist chamber for 12 hours after they were inoculated. He stated, however, that the disease was widespread in the field, showing particularly after moist weather, which seems to indicate that stomatal penetration took place. It is difficult to explain such a widespread occurrence of the disease if the organism enters only through wounds.

It is evident from Table 1 that in those instances where plants received a great amount of moisture before and after inoculation a high percentage of infection resulted, whereas a decidedly lower amount was observed when the plants were not given the moist treatment. Since covering produced a saturated humidity about the plant, a film of moisture formed on the exterior of the leaves and the substomatal cavities probably became well supplied with water. It seems reasonable to suppose that if moisture is a factor in influencing the movement of the stomata, there may have been set up a continuous passage of water from the exterior to the interior of the leaf; e. g., to the substomatal cavity. Thus, since the plants had received a previous moist treatment by being covered 24 hours before inoculation, the bacteria may have had a free swimming channel from the droplets of moisture that collected on the surface to the interior of the leaf and thus produced infection.

These experiments indicate that moisture is an important factor in favoring the production of disease. In Group 1, in which a high amount of moisture was present both before and after inoculation, the amount of infection ranged from very heavy to severe, whereas in Group 2, where abundant moisture was supplied only after inoculation, the amount of infection was somewhat reduced. In Group 3, where little moisture was present—that is, only that which was present in the greenhouse—very little infection was noted. These experi-

ments were repeated several times with a variety of plants and bacterial pathogenes, with similar results, indicating that moisture seems to be an important factor in producing infection by first influencing the movement of the stomata, which in turn allows a channel for the bacteria to make their way into the interior of the leaf. These experiments were performed at 10 a. m. and 5 p. m., with comparable results.

TRANSMISSION OF BACTERIAL BLIGHT

Considerable attention has been given to the problem of dissemination of bacterial blight because of the possible bearing it might have on control measures. Most of the evidence presented is observational, having been gathered for the last three years from experimental plots at Madison, Wis., from bean fields throughout that State, and from certain of the bean-producing areas of the western United States.

SEED TRANSMISSION

It has been known for a long time that bacterial blight is carried over from year to year in and on the seed. In this manner the disease spreads into districts that had previously been free from the malady. Beach (*3*) reported that probably the disease wintered over in the seed. Halsted (*16*) also inferred that infected seed transmitted the disease from one year to the next.

Both in the laboratory and in the field, little difficulty has been experienced in proving the existence of bacteria in the seed. The bacteria are harbored in the seed coats and also about and between the cotyledons, and when germination takes place they may either cause the death of the young hypocotyl before emergence or enter the cotyledons, causing vascular invasion of the plant. Cotyledonary lesions and the large water-soaked areas that appear on the first simple leaves and stems may serve as the initial sources of the secondary spread of the disease in the field.

During the summer of 1927 a study was conducted on the development of the disease under field conditions. For this purpose diseased seeds, alternated with healthy ones, were planted in several rows. When the bean plants were still small it was not a difficult matter to distinguish plants that were grown from diseased seed. Usually the plants were more spindling than normal ones, the cotyledons dried and dropped off before those of the healthy plants, and in many instances large angular water-soaked spots were to be seen on the first simple leaves. After a short period, certain of the plants surrounding such an infected seedling showed secondary lesions on their leaves. In the early part of the growing period only plants in the same drill row manifested these lesions, but later as the leaves enlarged the infection passed from row to row. It is evident that from the single infected plants serving as sources of inoculum the blight pathogene may spread very easily and cause severe destruction of a crop.

Most of the severely infected seeds are gleaned out by hand after threshing. It is difficult, however, to detect the slightly infected ones, since little shriveling or discoloration of the seed can be seen. These seeds when planted produce seedlings that serve as the initial sources for much of the secondary spread of the disease in the field.

Exudate is often seen oozing from stem or leaf lesions when conditions of high humidity prevail, as often exist during the early part of the growing season. From such sources rapid and widespread blight dissemination may take place.

OVERWINTERING ON BEAN STRAW

It was suggested by early workers that the disease may live over from year to year on infected vines and pods. Harrison and Barlow (*17*) stated that the bacteria can live over at least one winter in stems and leaves allowed to remain on the ground. McCready (*24*), without producing conclusive proof, stated that "the disease is carried over from year to year in the seed from a diseased crop, in the soil on which a diseased crop has been grown, or in straw from infected fields, in bedding or manure." Muncie's (*25*) observations are that the disease overwinters on diseased straw, and his experiments tend to prove this point, for the organism was isolated from diseased bean stubble which had remained in the field over winter.

The writer has gathered data from field studies which tend to lend evidence that the organism may live over the winter in this manner. There are, however, no experimental data to substantiate these observations. In one of the large bean-growing districts of Wisconsin serious outbreaks of the bacterial blight were in evidence during the summer of 1926. Wardwell Kidney Wax, one of the most susceptible of the commercial canning varieties, was being grown to a large extent that year. In a single field of about 8 acres practically 100 per cent of blight was estimated. After the bacteria had killed these plants cattle were allowed to feed on the diseased vines and the remaining stubble was plowed under. The following year the Improved Kidney Wax, a variety slightly less susceptible to bacterial blight than Wardwell Kidney Wax, was planted in this field about May 25, and on August 4 considerable blight infection began to appear. This circumstance would not have stimulated any thought of the overwintering of the organism if the other bean fields in the vicinity had shown any blight symptoms. It would naturally have been concluded that the infection was caused from diseased seed. Since, however, none of the other fields of this variety showed signs of the blight, although the seed was obtained from the same source, it was suspected that the bacteria might have overwintered on the diseased stubble and were then transmitted to beans planted in the same field the following year.

Similar observations were recorded in a number of the western bean-growing sections during the summer of 1928. During the summer of 1927 a large field of Full Measure beans was entirely destroyed by the bacterial blight, and about September 1 the diseased refuse was plowed under. In the spring of 1928 a large planting of beans was made in this same field and practically every variety grown was severely infected by August 1, and by the end of the month very few individuals could be found free from blight. High winds accompanied by rain and hail possibly may have accounted for some of the infection. But, since there were no bean fields in the immediate vicinity of the trials, and since the seed was grown in a section free from blight in 1927, it was suspected that much of the infection came from the diseased refuse that had been plowed under the previous fall.

INSECT TRANSMISSION

Whether or not insects transmit bacterial blight from plant to plant is unknown, but undoubtedly they play some part in the dissemination of the disease. Insects as spreaders of the blight have been reported by a number of workers. Sackett (*30*, *p. 212*) stated that "insects play an important part in disseminating the trouble, consequently any measures which tend to check these pests will aid in controlling bacteriosis." There is, however, no experimental evidence to substantiate these statements. In the present work the leaf hoppers (*Empoasca mali* Le B.), the 12-spotted cucumber beetle (*Diabrotica duodecimpunctata* Oliv.), and a ladybird beetle (species not identified) have been particularly noticeable feeding on the foliage of bean leaves.

It is possible that insects may carry the bacteria on their legs, bodies, and mouth parts and so become factors in disseminating the disease. At best they can not be considered as playing a major rôle in the dissemination of the disease, since its natural spread during favorable weather conditions is of primary importance.

DEW AS A FACTOR IN DISSEMINATION

Dew as a possible factor in the dissemination of bacterial blight was reported as early as 1901 by Halsted (*16*, *p. 15*), who stated that "it is not unlikely that the germs were carried from the diseased leaves to the pods by the dripping dews." Sackett (*30*) stated that "rain and dew are doubtless agents in spreading the germs from one part of the plant to another by washing them from old lesions onto unaffected parts." That moisture is essential for widespread infection became evident in both greenhouse and field studies. Heavy dews dripping from leaf to leaf may easily carry the pathogene and cause secondary spread of the blight. Many of the pod lesions found along the dorsal suture may be caused in this same manner. Dew which collects in droplets may run down the petiole, thence along the dorsal suture, carrying with it the bacteria that produce in many cases the characteristic vascular pod lesions. If the infection becomes established along the suture of the young pod, developmental growth often ceases and the pod shrivels and dies. When infection takes place after the beans have formed, they may easily become infected through the vascular connection of the seeds to the pod, and the pathogene can be carried over until the following year in this manner.

While the leaves are wet with dew the pathogene may spread from leaf to leaf if a thin film of water connects portions of the two leaves. It is for this reason that beans should not be picked or cultivated early in the morning or directly after a rain while they are still covered with moisture. If this is done, infection can be spread from plant to plant in the same row and even from row to row, in some cases causing destruction of the crop.

OTHER ENVIRONMENTAL FACTORS AFFECTING DISSEMINATION

That rain might be a possible factor in the dissemination of the blight was reported by many investigators. Rapp (*28*) states that following a rain accompanied by wind, bacterial blight spreads from

the center of primary infection to the greatest extent in a southeasterly direction. This, he says, is accounted for by the fact that wind-driven rain is blown in that direction and undoubtedly carries the pathogene from row to row, and in some cases across a number of rows. Observations made by the writer also indicate that splashing rains accompanied by winds are responsible for a great deal of the infection found in bean fields.

Hail is also very important in disseminating the disease from plant to plant. This was well demonstrated in many bean fields of the western bean-growing States. In the vicinity of the Greeley, Colo., project, much hail injury to crops was reported. Certain bean fields that were struck by hail showed almost complete destruction, much of the injury being caused by bacterial-blight infection. The dissemination apparently began from seedling-infected plants that supplied the source of inoculum. Because of the whipping of the leaves and the injury of the plants from the hail, the spread of the organisms was very rapid. A short time thereafter practically all the plants in the field were infected. Since comparable bean seed lots, planted in regions of hail injury and also in hail-free sections, showed a very decided difference in the amount of disease, it indicated that the hail and wind were the limiting factors in the dissemination of the disease. Fields well protected by trees, preventing to a great extent the whipping of leaves, also showed less disease than bean fields that were subject to high winds.

Some observational evidence has been accumulated regarding surface water as a means of spreading the bean-blight pathogene from diseased to healthy plants. These data were collected in 1928 at the bean trial grounds at Madison, Wis., where a portion of the field is sloping. During the early part of the growing season seedling infection near the upper portion of the field was recorded. After a series of heavy rains the plots were again visited, and the disease was more widespread, the direction of the spread being in many cases from old diseased centers. There was a tendency for the spread to be in the downward direction of the slope, either along the row or across the rows. In tracing these new infections it was observed that the pathogene had been washed from the diseased seedlings and carried down the small rivulets. Where this water laden with the bacteria came in contact with the plants, new infections took place. At least nine such instances were recorded, indicating that surface-drainage water very likely was responsible for carrying the organisms from diseased seedlings to healthy plants.

That irrigation water, which is used almost exclusively in the western bean-growing sections, may disseminate bacterial infection, came to the writer's attention on several occasions in a survey of many of those sections. It seems highly probable that the organisms may be carried down the small irrigation ditches and cause infection to other plants in the same row. Where infected seed had been planted, young lesions often extend to the ground level, and in many instances bacterial ooze has been seen exuding from such necrotic areas. Irrigation water in such cases may carry the pathogene from lesions to healthy plants in close proximity to the center of initial infection. Diseased leaves that had dropped from infected bean plants were often seen in these ditches. In this manner the organisms

could also be carried down an irrigation ditch, causing plants along the row to become infected.

THE PRESOAKING OF SEED AS A FACTOR IN DISSEMINATION

That water applied to seed in the inoculation with *Bacillus radicicola* Beij. spreads bacterial blight is very evident. Barss (*2*) remarks that beans should not be soaked in a liquid culture of *B. radicicola* for nodule inoculation, since the soaking method results in a general contamination of the entire seed lot, even if only very few seeds are infected. Leonard (*21*) has reported that a slight application of moisture will cause a stimulation of the bean-wilt disease, *Bacterium flaccumfaciens*. This was also noted by the writer in 1925 and 1927. Whiting, of the department of bacteriology at the University of Wisconsin, applied the wet-nodule seed treatment to a number of samples of Wardwell Kidney Wax variety. Apparently only a small portion of the original seeds was diseased, since few blighted plants were produced in the check plots. The treated seeds, however, produced plants manifesting approximately 100 per cent blight,[6] which were all killed before pod maturity. Since the checks produced few blighted plants as compared with the complete blighting of the treated seed, it was concluded that the dissemination was brought about by the wet-seed treatment.

In 1928 at Berlin, Wis., where the Full Measure variety was planted, a similar observation was made by the writer. Seed portions of this variety were treated with a liquid culture, whereas the remaining portion was planted without the treatment. A high percentage of the plants grown from the treated seeds were severely affected with typical cotyledonary symptoms, showing that the disease must have come from the seed. The untreated seeds produced plants showing a slight amount of infection, proving that the wet treatment accounted for the widespread occurrence of the disease.

RELATION OF PARASITE TO HOST

MATERIALS AND METHODS

The material for the investigation of the parasitic relationship of *Bacterium phaseoli* to the bean consisted principally of the Wardwell Kidney Wax variety. The material was collected both in the greenhouse and in the field. Before killing, portions of the diseased areas were plated out, in order to be positive that only *Bact. phaseoli* was present. Formal-acetic alcohol was used throughout as a fixing fluid. The sections were stained either with Giemsa stain (orange G as a counterstain) or with safranin (licht grün in absolute alcohol as the counterstain). These stains were used in a 2 per cent solution, and the sections were allowed to remain in them for a period of 6 to 12 hours, after which they were destained in absolute alcohol and then counterstained. These stains in the above dilutions were found to be very effective, because the host tissue took the stain faintly, and the differentiation between the bacteria and the surrounding tissues was very clear and distinct.

[6] Unpublished data from A. L. Whiting.

RELATION OF PARASITE TO LEAF TISSUE

Microscopic examinations, as previously reported by Smith (*36*), revealed that the organism gains its entrance through the stomata. Since stomata are more numerous on the under side of the leaves, it is here that the greatest degree of infection takes place. (Fig. 1, A and C.) Entry might also be made through wounds in the tissues. After the bacteria enter the stomata they pass into the substomatal cavity, multiply rapidly, and when in sufficient numbers penetrate into the intercellular spaces of the spongy parenchyma.

The bacteria appear to produce an enzyme which softens or possibly dissolves the pectic materials in advance of the pathogene. The middle lamella stains deep blue with the Giemsa stain and can be clearly differentiated from the primary walls of the adjacent cells. The cells in the vicinity of the infected area usually show abnormal characteristics. The lamellae of those cells take the stain more faintly than healthy ones, and in many cases it appears that portions are dissolved out, since there is no regularity in the intensity of the stain. Farther away from this region the cells are normal, and the lamellae take the stain very uniformly. In the region of severe infection the bacteria fill the intercellular spaces. This bacterial mass later becomes embedded in the slimy matrix, which causes an enlargement of the intercellular spaces owing to the absorptive powers of the slime. The epidermis remains intact, but the underlying parenchyma tissues collapse, often forming large bacterial pockets. When severe infection has taken place a large brown scaldlike area, due to the death of the cells below, appears on the leaf surface.

There appear to be two views as to how bacterial plant pathogenes cause the death of the host cells. Bachmann (*1*) believes that the cells are killed by the extraction of liquids from the protoplast followed by plasmolysis owing to the high osmotic pressure set up in the intercellular spaces incident to bacterial invasion. Another view by Steward (*37*) assumes that toxic products are secreted which diffuse into and kill the cells. The writer's histological studies seem to favor the theory of Bachmann. Staining reactions indicated that when the bacteria occur in large numbers they are always embedded in a slimy mass. The osmotic concentration of this material appears to be greater than that of the cell sap itself, and apparently an exosmosis takes place, causing the intercellular spaces to enlarge, and, as they become filled with a fluid, producing small water-soaked lesions on the leaf surface, characteristic of initial blight symptoms.

Surrounding these small water-soaked spots there develops a yellowish halo. Upon microscopic examination few bacteria are found in this discolored zone. In her study on the halo blight of oats, Elliott (*12*) found a similar condition. She stated that it is probable that the organism produces ammonia, which is responsible for the destruction of the chlorophyll about the lesions produced in oat plants. The cause of this discoloration in bean blight has not yet been determined, but it is believed that a toxic substance secreted by the organism diffuses into the surrounding tissue, causing the light-yellow zone.

Microscopic examinations have shown that the bacteria are commonly present in the xylem vessels of the leaf. (Fig. 2.) The

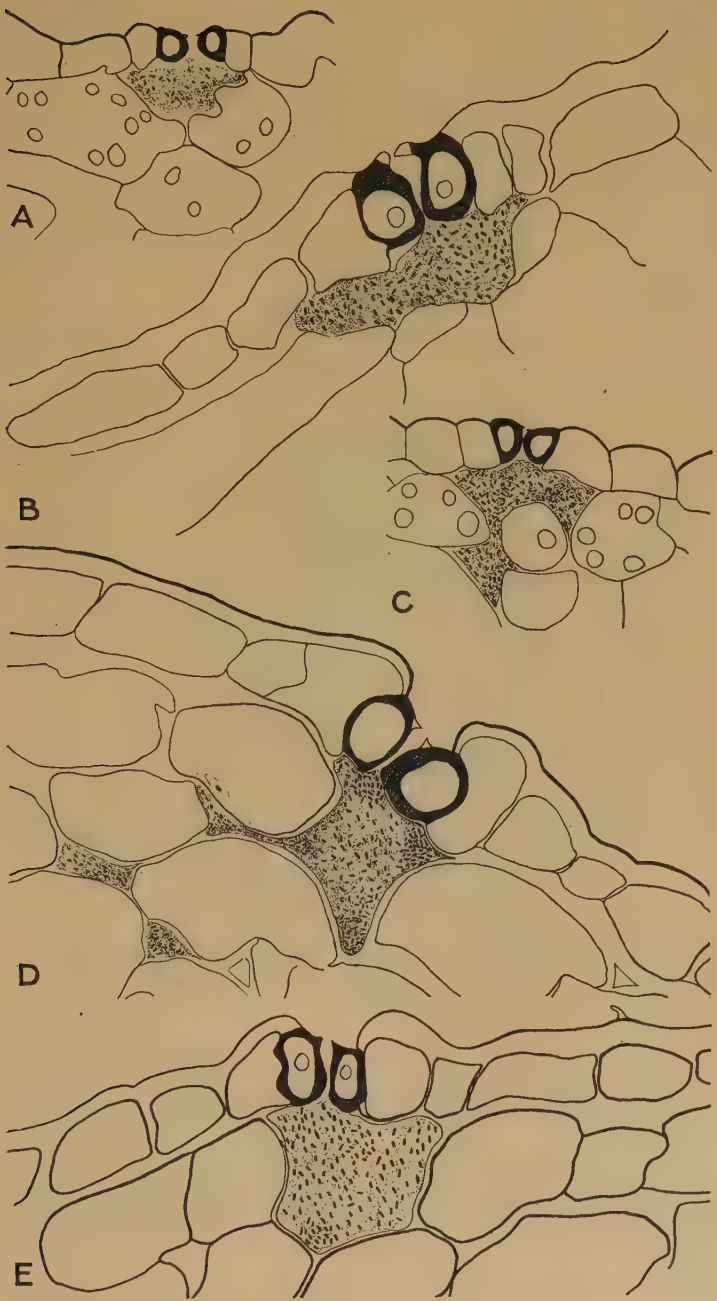


FIGURE 1.—Stomatal penetration by *Bacterium phaseoli:* Most of the penetration of the leaves, pods, and stems by this organism in nature is stomatal. A and C, Bacteria penetrating the stomata of the leaf. In each case the substomatal cavity is filled with a bacterial mass. In C the bacteria are following the intercellular spaces leading from the cavity. B and D, Bacteria invading stomata of the stem. E, Bacteria invading a stoma of the pod. × 1,250

organisms probably enter the large xylem vessels by first invading the small veinlets, which in their initial stages of development consist of undifferentiated tissue similar to that of the surrounding parenchyma. These tissues appear to be easily attacked, and after the organism once gains entry it passes into the large veinlets, which in turn lead into the main veins of the leaf. After gaining access into this tissue the bacteria multiply rapidly and when in sufficient numbers cause a browning of the veins and veinlets (pl. 1, C) with a gradual killing of the surrounding tissue. The pathogene might also enter the vessels of the leaf by passing from infected petioles

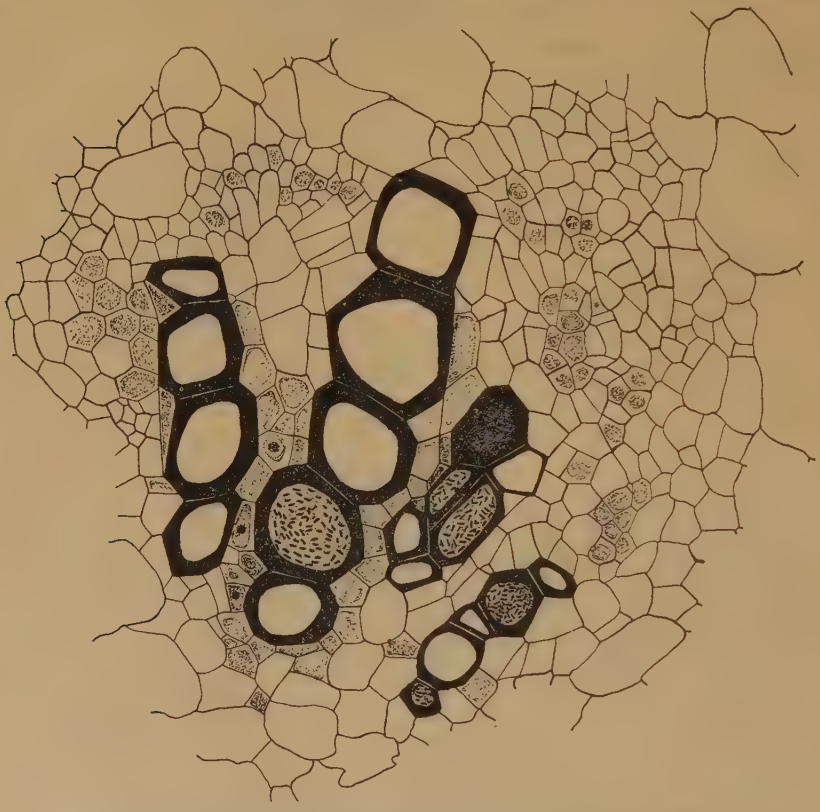


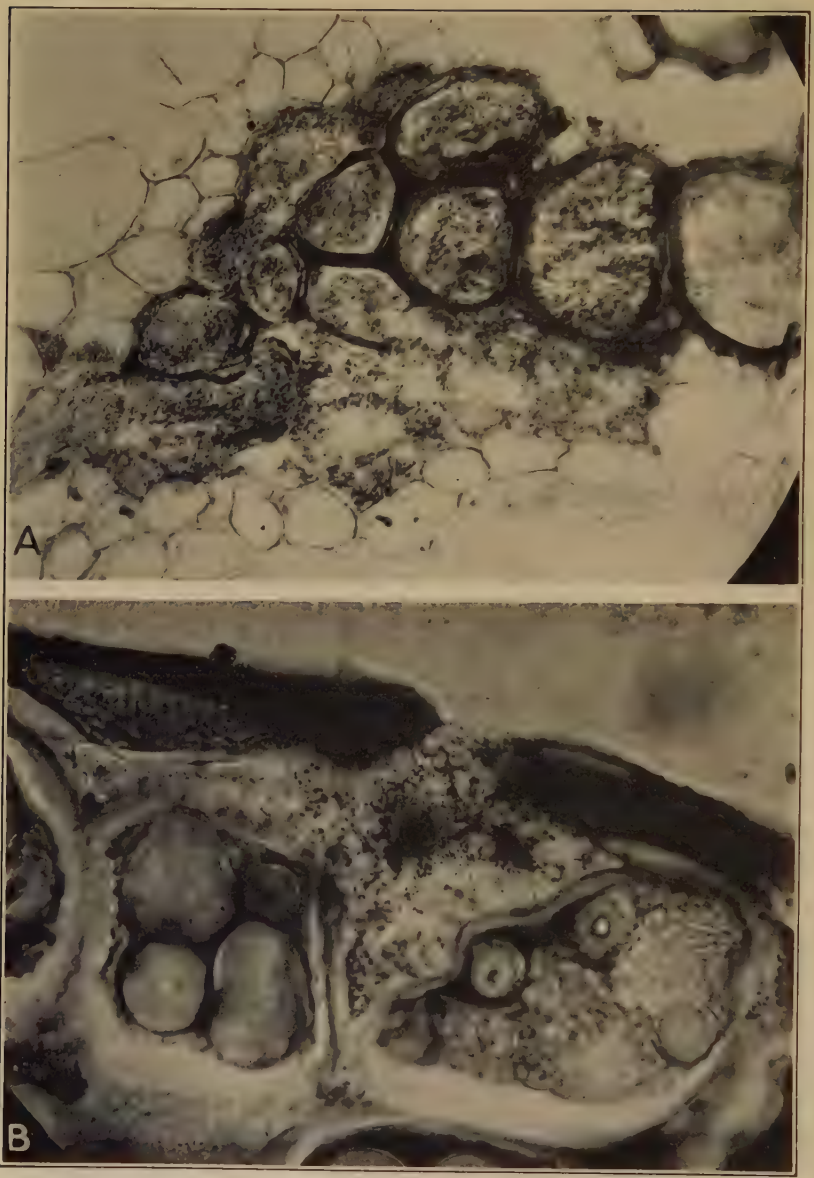
FIGURE 2.—Vascular invasion of a bean leaf. Cross section of a midrib showing bacteria embedded in a slimy matrix in the xylem vessels. × 1,250

through the pulvinus and into the main vein. The bacteria seem to become localized in the pulvinus, possibly because of the succulence of this tissue. A severe invasion of these structures results in a drooping of the leaves, a very characteristic symptom of primary seed infection.

RELATION OF PARASITE TO STEM TISSUE

Burkholder (*5*) and Barss (*2*) observed that the disease was systemic in nature. Microscopic study revealed that the vascular system of the stalks was invaded by the bacteria, although no external lesions appeared on the leaves or pods.

A, Bacterial exudate (*a*) on bean stem, 10 days after inoculation, × ⅔; B, infected bean seedling showing drooping of leaves at pulvinus (*a*) and stem cracking (*b*) owing to bacterial infection, × ½; C, darkened veins and veinlets (*a*) following invasion of the vessels by the bacteria. Small water-soaked lesions (*b*) result from stomatal infection and may result in further vascular invasion when in contact with veinlets, × 1; D, bacteria have encircled the epicotyl and produced a girdle (*a*) which caused the stem to weaken. Natural infection, × ½; E, infected bean stem showing longitudinal red-colored lesions caused by bacterial infection. Natural infection, × 1; F, pod lesions resulting from stomatal infection. An incrustation of dried bacterial slime is seen in the center of many spots. Natural infection, × ¾; G, diseased seed of the Bountiful variety showing shriveling and discolorations caused by bacterial invasion of the seed coats. Natural infection, × ¾; H, seeds from pod shown in I. The three shriveled seeds at the left were removed from the extreme right portion of the pod, × ¾; I, pod of the Bountiful variety showing discoloration along the dorsal suture due to bacterial invasion, × ¾



PHOTOMICROGRAPHS SHOWING BACTERIAL INVASION OF THE HYPOCOTYL AND COTYLEDON

A, Bacteria in the xylem vessels of the hypocotyl. In some cases they have broken out from the vessels and are causing a disintegration of the adjacent tissue, forming bacterial pockets. × 1,034; B, bacteria entering an epidermal rift of the cotyledon caused by a stretching of the cells during germination. The pathogene can be seen following the intercellular spaces, causing them to enlarge and the cells to be pushed apart. The adjacent cells are in a distorted condition from a pressure exerted by the bacterial slime in the intercellular spaces, × 1,034

Artificial inoculations were performed by the writer by cutting off the young cotyledons before the abscission layer had formed and inserting a drop or two of bacterial inoculum into the cut. This allowed the bacteria to enter the vascular system, and within a period of 10 days the inoculated plants manifested slight symptoms of wilting. The check plants appeared normal in all respects. Isolations from such material and also from wilted seedlings grown from diseased seed demonstrated the presence of the bacteria in the tissues. The pathogene was found through microscopic examination to be present in great numbers in many of the xylem vessels (figs. 3 and 4) and to extend up and down the stem from the cotyledonary nodes. In severe cases of infection the bacteria appear to break through the walls of the invaded vessels and to spread into the near-by paren-

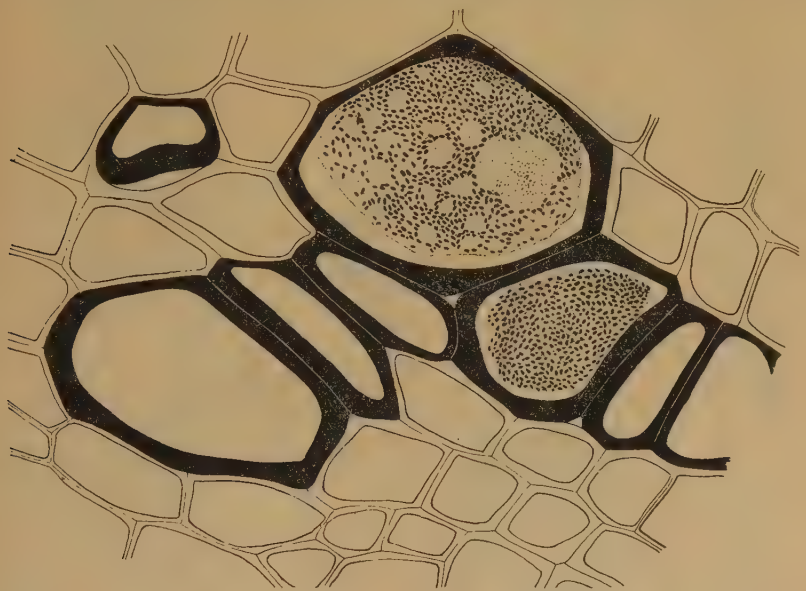


FIGURE 3.—Cross section of a bean stem showing invasion of metaxylem vessels by bacteria embedded in slime. × 1,250

chyma cells. (Pl. 2, A.) As stated before, in the growth of these organisms much slime having the property of absorbing a large amount of fluid is always produced. With this absorption a natural internal pressure may be set up, but whether this pressure is great enough to cause a rupture of the cells is still undetermined. When the bacteria break through the cell walls they enter the intercellular spaces of the adjacent tissue (fig. 5, A, B, and C), slowly dissolve the middle lamella of these cells, and finally push them apart with a gradual disintegration of the tissues. (Pl. 2, A.) Bacterial cavities are not uncommon in such regions. That the pathogene may enter the stem from infected leaves appears to be very probable.

In many cases where local infection begins through stomata in the vicinity of small veinlets which are found throughout the leaf, the readily attacked xylem elements of these veinlets are occupied by

bacteria. After the bacteria enter the small veinlets they make their way into the larger veins and thence into the midrib, traveling down the petiole and into the stem of the plant. The bacteria may also enter the xylem vessels of the stem by way of the cauline stomata. (Fig. 1, B and D.) The bacteria entering these openings multiply in the substomatal cavities and later penetrate the intercellular spaces of the cortical cells. Within a comparatively short time the pathogene may have invaded much of the surrounding tissue, producing a water-soaked lesion which manifests itself on the exterior of the

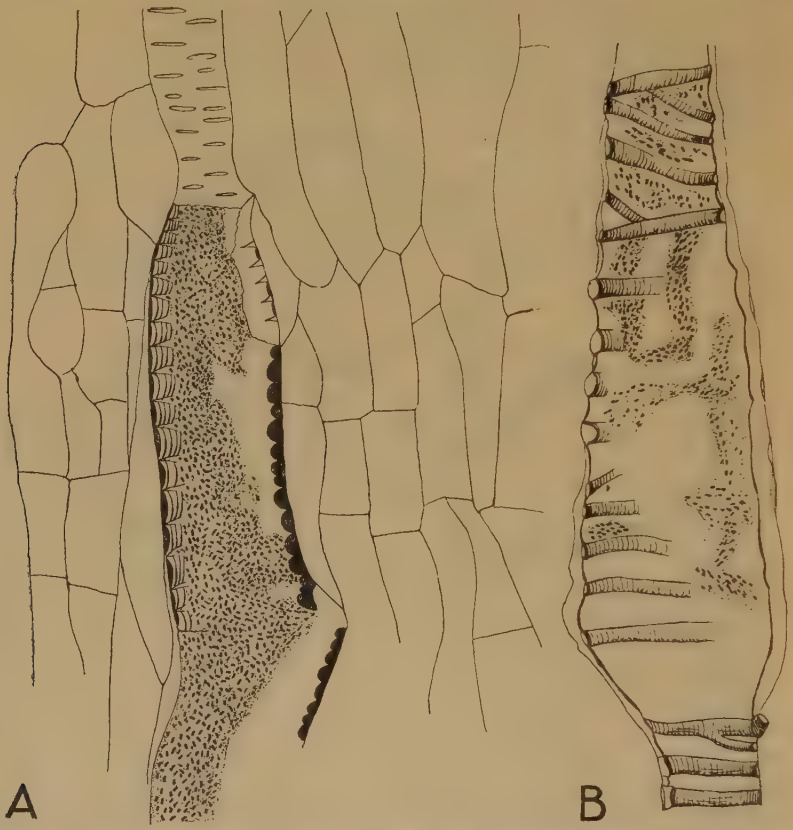


FIGURE 4.—Bacteria in xylem vessels of a bean stem: A and B, Bacteria embedded in a slimy matrix invading ringed metaxylem vessels. × 1,250

stem. When in sufficient numbers the bacteria begin to cause disintegration of the invaded cortical tissue, forming in many cases large lysigenous cavities.

In numerous sections in which penetration has begun from the exterior of the stem the cortical tissues are severely invaded by the pathogene, but within the endodermis few bacteria are in evidence. The layer of cortical cells contiguous to and including the endodermis appears to act as a barrier in partly preventing the bacteria from penetrating into the vascular tissue. (Fig. 6.) These cells do not completely surround the stele of the stem, and it is through the

breaks that the bacteria make their way into the xylem vessels. Such penetration takes place only when the plants are young—that is, before secondary thickening has taken place—since the cells of the secondary xylem appear to be little affected by the bacteria. The cells of the endodermis are very thick walled, with small intercellular spaces. It is possible that because of this adaptive structure the organisms are unable to penetrate this tissue.

Bacteria are seldom if ever found in the phloem region; most of them invade the protoxylem and metaxylem cells (fig. 3); only occasionally are they found in the secondary xylem. It is probable that in the formation of the cell walls of the primary tissue the wall materials are built up gradually, which means that lignification is the

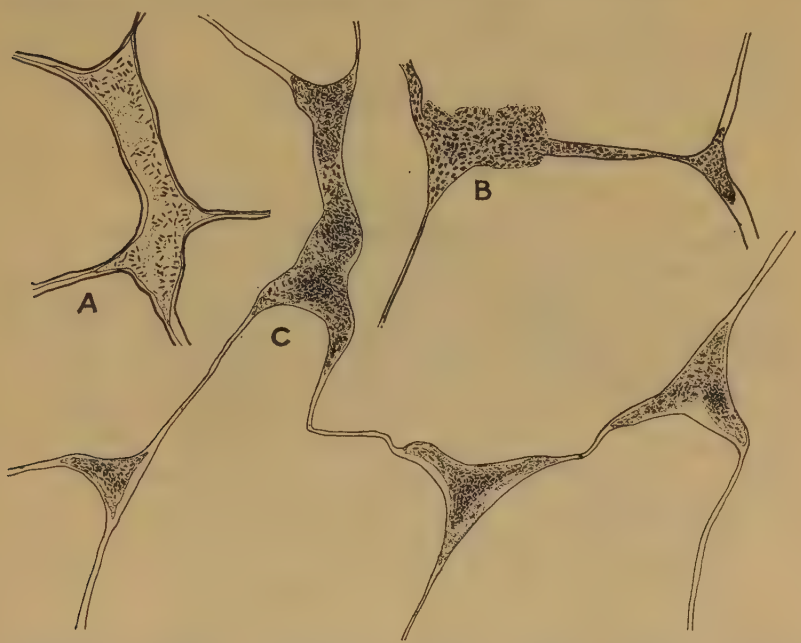


FIGURE 5.—Intercellular penetration of parenchyma cells by bacteria: A and C, Enlarged intercellular spaces due to invasion by bacteria embedded in a slimy matrix; B, portion of a cell wall disintegrated through bacterial action, allowing bacteria to enter adjacent cells. × 1,250

last process. The cells in the early stages of growth contain large amounts of cellulose or of hemicelluloses. These substances most likely are attacked by bacterial action, and entrance may be gained into the young protoxylem and metaxylem cells. However, in the formation of secondary xylem, which is laid down rapidly, the walls possibly remain in the cellulose state for a very short period, becoming lignified very rapidly. It is probable that the principal reason that little penetration occurs in this tissue is because of the wall composition of the cells.

That the bacteria together with slime resulting from rapid multiplication of the organisms plug the vessels and cause a wilt of the plant is not an established fact. Burkholder (*4*) believes that under

certain conditions the bacteria may enter the vascular tissue without causing a permanent wilting, but may persist, causing dwarfing, and on days of high evaporation may produce a slight flagging of the plant. According to the writer's investigations, little of the wilting of mature plants caused by *Bacterium phaseoli* appears to be due to plugging, but more likely it may be accounted for by a disintegration of the invaded tissue or the effect of toxic substances produced by the parasite. Microscopic examinations have never revealed that infected xylem vessels were so completely filled with bacterial masses that death may have resulted from a plugging of

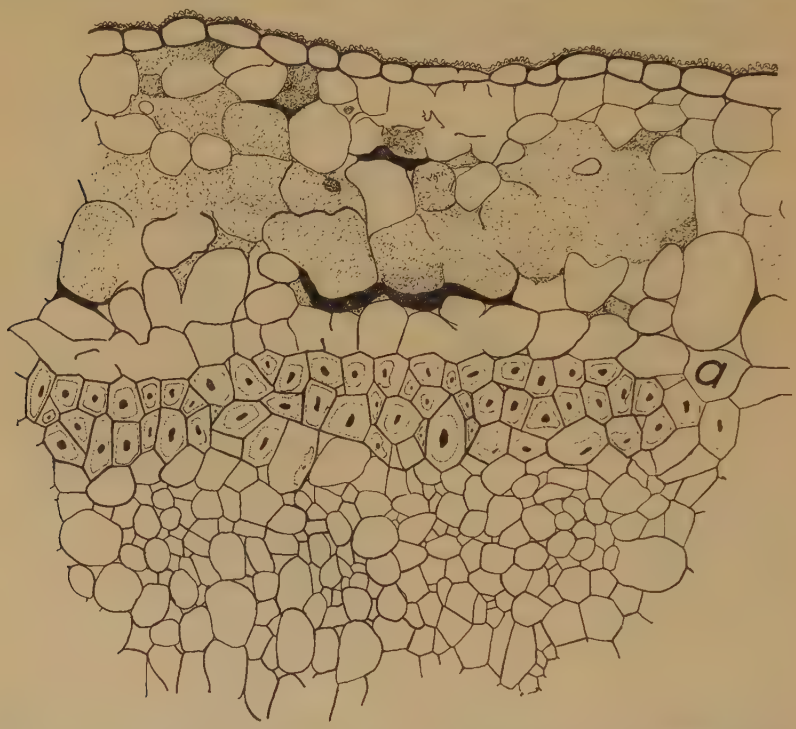


FIGURE 6.—Bacterial invasion of the cortex of the hypocotyl. Cross section of the hypocotyl, showing bacteria invading the cortex where a layer of cells (*a*) adjacent to the endodermis appears to act as a barrier to invasion of the vascular tissue. × 1,250

these cells. Since secondary xylem is formed rapidly in the stems of older plants, the passage of water would not be sufficiently hampered to cause wilting, even though the protoxylem and metaxylem cells were filled with bacterial masses.

The bacteria may enter the xylem vessels of the hypocotyl and epicotyl from the infected cotyledons in the case of diseased seed. Under favorable conditions the pathogene may increase with such rapidity that the vessels become filled before much secondary thickening has taken place. In this case a plugging of the vessels may bring about a wilting of the young plant.

CELL-WALL DISINTEGRATION THROUGH BACTERIAL ACTION

Smith (*34*), working with *Bacterium campestre* on turnip, stated that the infected cells are crowded apart by the growth of the bacteria, and the middle lamella first disappears, but the cell walls proper also become vague in outline and finally disappear. Microscopic examinations have shown that in the case of severe infection of the hypocotyl, epicotyl, and funiculus with *Bact. phaseoli*, disintegration of wall material becomes apparent. Careful observation with serial sections indicated that the microtome knife was not the cause of the disappearance of the wall or parts of it. (Pl. 2, A, and figs. 7 and 8.) It appeared that the cellulose walls became disintegrated, leaving only the lignified portions behind.

The protoxylem and metaxylem cells of the hypocotyl and epicotyl in which most of the infection occurs differ markedly from the secondary xylem cells of the same tissue in cell size and in wall structure. Since the protoxylem cells are formed very early in the

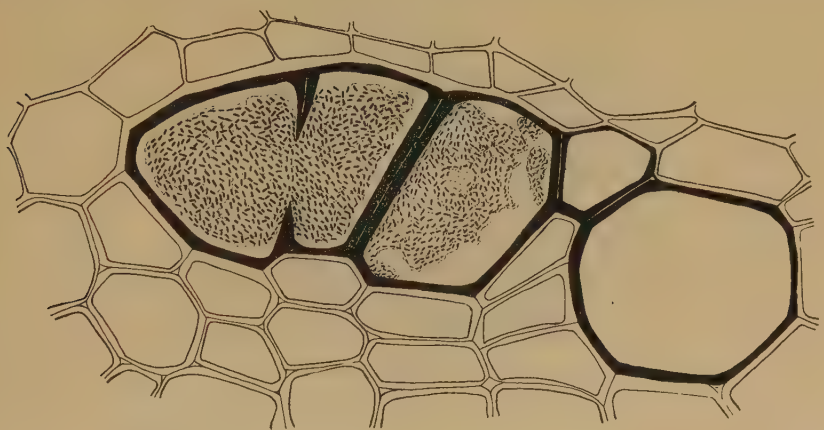


FIGURE 7.—Cross section of a bean stem, showing bacteria invading metaxylem cells and passing from cell to cell through broken wall. × 1,250

ontogeny of the tissue in which they lie, they are subject to tissue stresses from increase in length and diameter. These protoxylem cells mature rapidly and are not subject to growth changes. The stresses brought about by elongation tend to stretch the already mature cells, and in many cases a rifting results. These cells are long and slender, with thin cellulose walls, reinforced by bands of lignified secondary walls to prevent the collapse of the thin plastic walls. If bacteria are present in such cells, it is probable that natural rifting of the tissue would give the appearance of disintegration; but since this occurs only in the protoxylem, it does not explain the partial disappearance of the walls in many of the severely infected metaxylem cells.

The water-conducting cells of the metaxylem are the characteristic cells of this tissue because of the peculiar adaptation of their walls to the stretching that they normally undergo. The thin plastic primary walls of these empty cells are also strengthened by the addi-

tion of a lignified secondary wall. The metaxylem cells first formed have small amounts of secondary wall in the form of rings, whereas cells formed a little later possess spiral bands. The proportionate amount of secondary wall increases in the successively formed cells.

The fact that the greatest amount of infection occurs in the primary xylem may possibly be explained by the chemical composition of these tissues. Since the walls of the protoxylem and metaxylem cells are composed mostly of cellulose, with only small amounts of lignin in the form of spirals or rings, bacteria when in large masses probably have the ability to produce an enzyme (cellulase) with the property of slowly dissolving the materials between the pits of the vessels and finally other portions of the cellulose walls, thus making

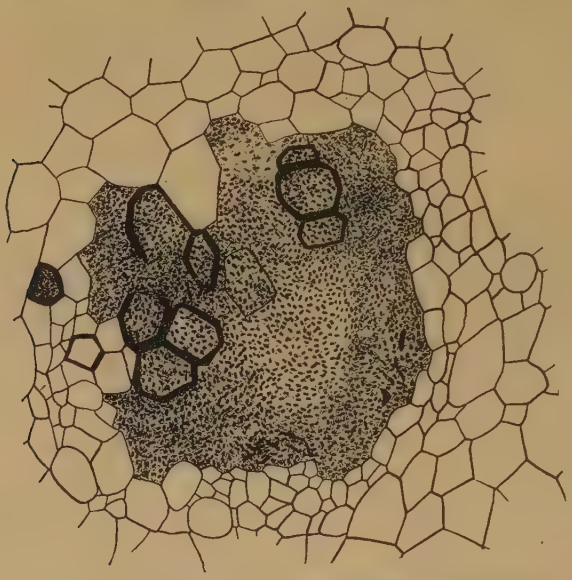


FIGURE 8.—Cross section of a bean funiculus, showing bacterial disintegration of tissue. The bacteria have invaded the xylem elements of the funiculus and disintegrated the tissue with the formation of a large lysigenous cavity in the lower part of which portions of the disintegrated walls may be seen. × 1,250

it possible for the pathogene to gain access directly into the xylem elements or pass from one vessel to another. Little infection takes place in the secondary xylem cells, probably because the walls are composed mostly of lignin, which the pathogene apparently is unable to attack.

In the funicular region of infected pods other instances of cell-wall disintegration were in evidence. Microscopic examinations of invaded funiculi many times revealed large lysigenous cavities showing many stages of cell-wall disappearance. (Fig. 8.) The walls of many of the invaded cells were thinner than the normal walls; others were slightly visible, whereas many of them had disappeared altogether. Scattered throughout these large bacterial cavities were strands of lignified wall material not attacked by bacterial action.

RELATION OF THE PARASITE TO PODS AND SEEDS

It is a well-established fact that *Bacterium phaseoli* is seed borne. One of the most important points in the behavior of the organism is its ability to enter the pods through the vascular system and infect the seeds without causing lesions on the surface of the pods. In entering the seed through the vascular system the pathogene frequently causes only a small yellow discoloration at the hilum. (Pl. 1, G.) This sign of the disease is not readily detected in colored seeds, as is also the case in white seeds, where normally there is a slight yellow marking about the hilum. Seeds that possess only a small amount of infection without outward symptoms may cause much damage when planted the following year. From the standpoint of control vascular seed infection is extremely important. It becomes apparent that the selection of pods is not an adequate means of control as in the case of bean anthracnose, where the infection is localized. To be positive that only disease-free seeds are obtained, only pods from healthy plants should be selected, and even then, if vascular infection is slight, apparently healthy plants may produce seeds that harbor the pathogene.

In causing this type of vascular infection, the bacteria travel up the xylem vessels of the stem, through the vascular elements of the pedicel, whence they pass into the two sutures of the pod. The lesions on the sutures of severely infected pods are easily detected, since they cause a discoloration of the vascular tissue, and in many cases water-soaked lesions extend along these sutures, particularly the dorsal. (Pl. 1, I.) The bacteria then travel from the xylem vessels of this suture to those that pass into the funiculus and are then carried into the seed coats by way of the raphe. The raphe extends only a short distance into the integuments and becomes undifferentiated tissue, similar to that making up the third and fourth nutritive cell layers of the seed coat. After the bacteria enter the coats they make their way into the intercellular spaces, which are extremely large and afford an easy passage for the spread of the organism throughout the tissue. (Fig. 9, A.) When in sufficient numbers the organisms may destroy this tissue without the production of any outward symptoms on the seed.

The first layer of the seed coat is composed of large palisadelike cells, thick walled and upwards of 60μ in length. On both sides of the hilum slit, which is found in the center of the hilum, two layers of palisade cells are present, while immediately beneath the slit is a group of sclerenchyma cells with reticulated walls which, according to Tschirch and Oesterle (*38*), probably serve to prevent the entrance of fungi into the seed. Below this palisadelike epidermal layer is found a layer of cells known as the "I" layer, being made up of hourglass-shaped cells 18μ to 22μ in height, containing calcium-oxalate crystals and possessing no intercellular spaces. Below these two layers are found the nutritive spongy tissue with large intercellular spaces into which the raphe enters. Bacteria passing into the seed coats by means of the raphe traverse this region extensively, as previously mentioned, but they seldom invade the first two layers of the seed coat.

The old conception that direct penetration occurs through the outer layers of the seed coat is difficult to explain, since in the first

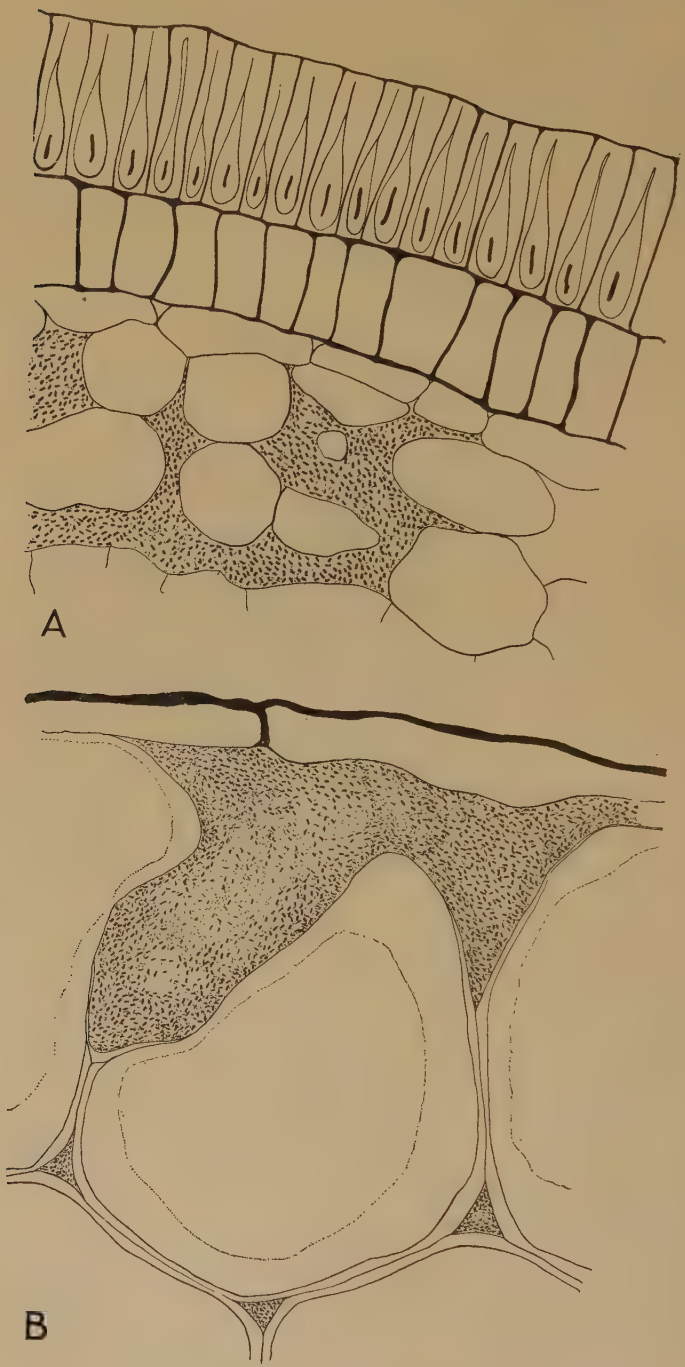


FIGURE 9.—Intercellular penetration of bacteria in bean-seed tissues: A, Bacteria are shown in the large intercellular spaces of the third layer of the seed coat; B, bacteria embedded in slime in the cotyledonary intercellular spaces, causing the cells to be pushed apart. × 1,250

place the epidermal cells are covered with a layer of cutin or suberin; and secondly, their cell walls are extremely thick and without intercellular spaces so that penetration would be difficult. The symptoms on mature seeds infected other than at the hilum, which are so often illustrated, may be caused by some other parasite, possibly *Colletotrichum lindemuthianum*. Much of the discoloration seen on mature bacterial-blight infected seeds is probably due to the disintegration of the cells below the palisadelike epidermal cells. This is especially apparent in white-seeded varieties. In severely infected seeds, besides this discoloration, a shriveling occurs, because of the collapse of the third, fourth, and fifth layers of the first integument. In cases where bacterial penetration is slight, no discoloration is in evidence at the hilar region, and it is often impossible to demonstrate macroscopically the presence of the organism, although if severe infection takes place a yellow or water-soaked discoloration is evident.

When bacteria are found in large masses in the vascular elements of the dorsal suture of the pod, they often cause disintegration of the tissue and enter the surrounding parenchyma cells and thence pass into the pod cavity where the young ovules are beginning to develop. When funicular infection is severe, this tissue is destroyed, and the seeds fail to undergo further development. It is a common occurrence to find one or two ovules of a pod decidedly shrunken, with the remaining seeds developed to maturity.

When the pathogene enters the seed while in the milk stage the bacteria in the seed coats may pass into the regions of the cotyledons and under certain conditions entirely surround these structures. The bacteria appear to remain in this region as well as in the seed coats in a dormant condition and do not cause the embryonic plant to become infected until the time of germination.

This type of infection has been reported by both Barss (*2*) and Burkholder (*5*) as being of a serious nature, since no external lesions are noticeable on the pod. The writer's researches have shown that a considerable amount of infection takes place in this manner; however, bacterial penetration through the micropyle of the seed is equally as important as vascular penetration and possibly more widespread.

The bacteria may enter the pod cavity, as stated above, by breaking out from the vascular tissue of the dorsal suture or the funiculus, or by making their way into the pod stomata. From here, as in the leaf and stem, the pathogene fills the substomatal cavity (fig. 1, E), passes into the intercellular spaces, gradually becomes intracellular, and later causes a disintegration of this tissue. The organisms have likewise been found in the xylem vessels, which may distribute them to all portions of the pod. In some cases they break out from the vessels and when in large masses cause disintegration of the surrounding cells. They may then pass into the pod cavity, where they increase rapidly because of the favorable conditions for their development. Numerous pods have been cut open, and in many instances where only a slight amount of infection was in evidence on the exterior of the pod the inner cavities contained large masses of bacteria embedded in a slime, surrounding, in many cases, each individual seed. In such cases micropylar invasion is a simple matter.

The micropyle is a natural opening in the palisade epidermis of the seed coat, and the bacteria may enter here and reach the seed coats with little difficulty. Below this point of entry a cavity is found, and the surrounding cells, which are decidedly thick walled, appear to be little affected by bacterial action. However, when the organisms once make their way into the underlying tissues, little opposition seems to be encountered. The bacteria pass readily through the large intercellular spaces of the seed coat (fig. 9, A) and later break down this tissue, causing in severe cases a shriveling of the developing ovule.

It is in the immediate vicinity of the micropylar opening that the young developing embryo is found. As the seed begins to germinate, the young hypocotyl elongates and may push its way through this diseased region as it emerges through the seed coat. Since the young epidermal cells are very compact, with small intercellular spaces, penetration into this tissue appears to be somewhat difficult.

In examining diseased germinated seeds it is not uncommon to find the embryonic tip of the hypocotyl of many young embryos killed before emergence. It seems likely that the pathogene present in the seed coats in close proximity to this structure may cause the death of these tissues, but the entrance of the bacteria into these cells while they are still in the embryonic condition has not as yet been actually observed. If the bacteria are later found to enter these cells, then much of the seedling wilt can be explained.

Stomata have been found on the hypocotyl above and below ground after cell elongation has taken place. As this structure grows through the diseased seed coat, it appears to be possible for the pathogene to be carried along the surface of the cells, and as the stomata are formed the adhering bacteria under favorable conditions might enter these openings, causing infection in this region.

It has likewise been observed that often in the case of diseased seed the embryonic folds of the epicotyl, while still lying between the cotyledons, are surrounded by bacteria. This fact may account for the occurrence of the common initial water-soaked lesions often found on the primary leaves of young diseased plants. These lesions usually appear on the opposite simple leaves in exactly the same position, making it appear that the bacteria enter these embryonic structures at the time when the primary leaves are still folded together.

The bacteria that pass into the micropyle and invade the large intercellular spaces of the seed coat may remain there in a dormant condition until they penetrate into the cotyledonary tissue at germination time. On the other hand, if they enter the seed through the vascular system they become well established in the seed coat also, since they multiply therein and pass throughout by way of the intercellular spaces. (Fig. 9, A.) In this way they may migrate into the region of the micropyle, at which point they may pass out of it and enter the cavity of the pod, where they multiply rapidly because of the extremely high moisture conditions and abundant food supply. The organisms spread readily throughout this cavity and may infect other young seeds by entering their micropyles. In this manner it can be seen that from a single infected seed all the seeds of a pod may become infected without the presence of a lesion on the exterior of the pod.

PENETRATION OF BACTERIA INTO THE COTYLEDON

The most important phase of seed infection is the penetration of the organism into the cotyledonary tissues. For the study of this phase of the problem, diseased seeds were surface sterilized in a solution of mercuric chloride 1–1,000 for 35 to 45 seconds, washed in three changes of sterile water, and placed in sterile Petri dishes between moistened sterilized filter paper. The seeds were allowed to germinate, and after the young hypocotyl had emerged through the micropyle the seed coats were removed and one of the cotyledons of each seed was killed in formal-acetic acid fixative, the other being used for the isolation of the organism. The killed cotyledons were embedded in paraffin, sectioned, and stained in the usual manner.

Since cotyledonary penetration occurred only in germinated seeds, it was suspected that the reason for the lack of infection in ungerminated ones was because of some protective covering, either cutin or suberin, over the cotyledonary epidermal cells of ungerminated seeds. Microchemical tests were made for the presence of these substances by treating freshly cut sections of cotyledons with a solution of Sudan III. The sections were allowed to remain in this solution for 15 minutes, after which they were washed in 50 per cent alcohol, placed in glycerin, and examined. The tests demonstrated that the amount of suberin or cutin was extremely small and that there was no difference in the amounts in the cotyledons at various stages of development.

Microscopic examination of the embedded material revealed that as the cotyledon absorbed water at the time of germination the sudden enlargement of the epidermal cells resulted in numerous instances in the pulling apart of their adjoining outer walls. After observing these small rifts, and in many cases large tears, in the cotyledonary tissues, measurements of epidermal cells were made before and after germination. These cells are distinctly of two sizes, those adjacent to the hilum being considerably longer than the cells on the opposite side of the cotyledon. Measurements of 50 cells of both types were made before and after germination. It was noted that the average length of the former cells before germination was 46μ, whereas these same cells after germination had enlarged to approximately 82.8μ in length. The other type of cells before germination averaged 8.64μ, whereas after germination they increased to 14.25μ in length. As for increase in width before and after germination, little difference in size was noted.

It becomes apparent that with the imbibition of water the seed swells enormously. The mature cell walls apparently do not allow for much expansion, and because of the enormous increase in length a natural pulling apart of the cells results. (Pl. 2, B, and fig. 10, B, E, and F.) Some of these tissue tears are extremely small and are often in the form of a small V (fig. 10, B), while others have been found to measure as much as 9.8μ.

With the pulling apart of the epidermal cells there is likewise a stretching of the intercellular spaces. The bacteria on the outside of the cotyledon may enter these rifts, pass into the intercellular spaces, and in some cases cause them to swell to enormous size with the distortion of the adjacent cells. (Pl. 2, B, and fig. 9, B.) Disintegration then takes place, and the bacteria rapidly traverse the

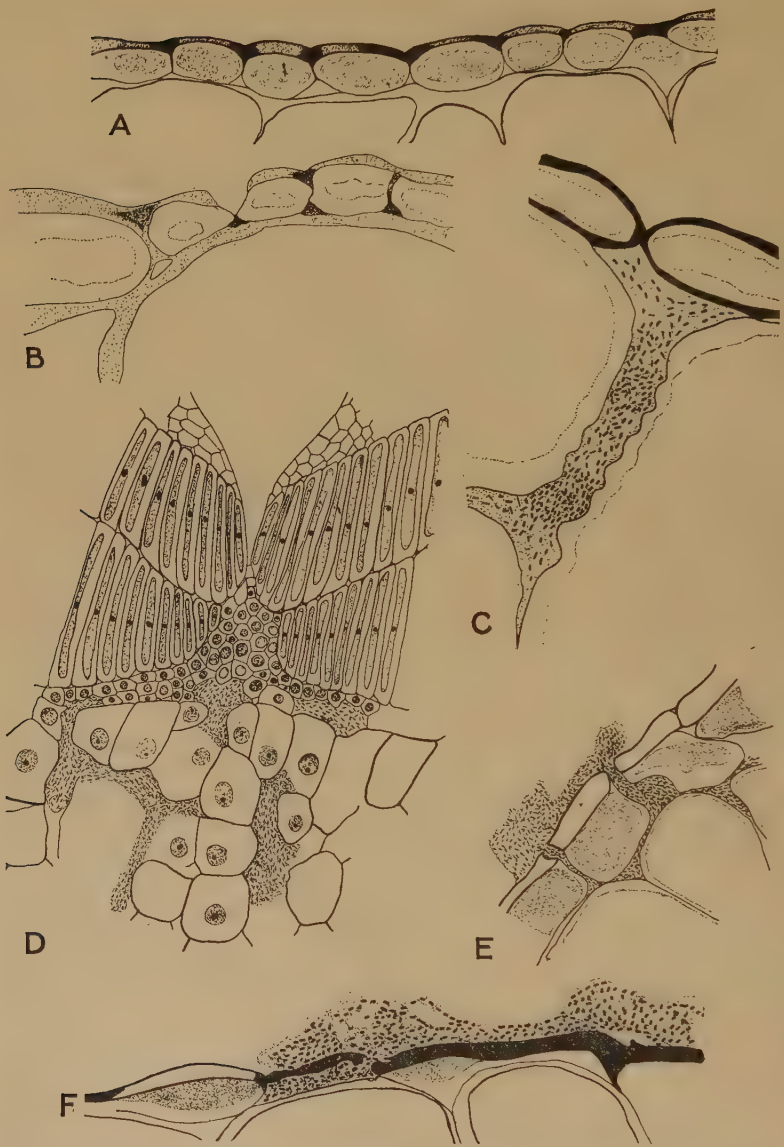


FIGURE 10.—Cross sections of bean-seed material showing normal and bacteria-infected tissues: A.—Normal cotyledonary epidermal cells which show dark-stained areas where the cells are pulled apart at germination time. × 1,250. B.—A natural rift in the epidermis caused by a stretching of the epidermal cells. × 1,250. C.—Bacteria embedded in a slime in the intercellular space of cotyledonary cells. × 1,250. D.—Bacteria invading the cells of the funiculus. × 850. E. and F.—Bacteria entering the natural epidermal rifts of the cotyledon and infecting the cells below the epidermis. × 1,250

cotyledonary tissue, often forming cavities. It appears that the bacteria may then pass into the vascular elements and thence enter the xylem cells of the hypocotyl and epicotyl at the cotyledonary node.

In connection with epidermal penetration, microchemical tests were made to determine the possible composition of the cell walls at various stages of development. Fresh sections of seeds in the milk, mature, and germinating stages were made and placed in small vials of ether for the extraction of existing oils and fats. The material was then treated with a 0.05 per cent solution of ammonium oxalate and placed on a sand bath at 90° C. for a few hours. The vials were allowed to stand for a short period at ordinary temperatures, and upon examination it was observed that the cells of the germinated cotyledons were not well intact and apparently had their middle lamellae somewhat dissolved by the ammonium oxalate. The epidermal cells were likewise in a disintegrated condition, demonstrating that the walls were probably composed of a soluble substance, pectinlike in nature. The walls of the cotyledons in the milk stage and also those in the mature condition remained intact, demonstrating the presence of an insoluble substance.

From this it can be assumed that penetration into the germinated cotyledon might be explained by a dissolution of the soluble material in the walls of the cotyledonary cells. At the time of germination the food materials in the seed are being changed to soluble substances for the nourishment of the young developing embryo. If the composition of the epidermal cells is likewise changed, as the foregoing experiment indicated, it appears that the bacteria, after once gaining entrance into the epidermis through small ruptures that are formed during germination, make rapid progress into the inner tissues of the seed.

Further to substantiate the above results, similar material was stained with the Giemsa stain, which, as stated before, has an affinity for middle-lamella material, staining these substances deeply. Certain regions of the epidermal walls of uninfected cotyledons took this stain very readily. (Fig. 10, A.) These regions were found between the cells and extended a short distance to both sides of these cells where bacterial penetration in the case of diseased cotyledons took place. It seems reasonable to suppose that these dark-stained areas are composed of a soluble pectinlike substance, as the results indicated. If, as is believed, the bacteria secrete an enzyme, pectinase, these darkly stained areas can be broken down, especially after the bacteria have made their way into the small epidermal rifts caused by an enlargement of the cells. (Fig. 10, F.)

Briefly, the cycle of development of the disease in the light of the pathological histology may be traced as follows: The bacteria in the seed coats make their way into the region between and about the cotyledons, and with the germination of the seed the pathogene enters the cotyledonary tissue, follows the intercellular spaces (fig. 10, C), and finally may gain access to the vascular elements. From here it passes into the young seedling, traveling up the vessels of the epicotyl and part way down the hypocotyl. Burkholder (*5*) states that the bacteria extend into the root system. He remarks that in the xylem vessels of the tap and lateral roots great masses of bacteria are found similar to those observed in that part of the plant above

ground. Similar results have not been observed by the writer; however, bacteria have been demonstrated in the vessels of the transition stage of the hypocotyl, that portion in which the xylem development changes from the exarch to the endarch condition.

After the bacteria enter the vascular system the seedling often wilts. The cause of this wilting has not been definitely established as yet. It might be owing to a plugging of the vessels by the bacteria and retarding the transpiration stream of the plant by causing the invaded tissues to become disintegrated or as a result of toxic effects of the metabolic by-products of the organism. The bacteria in the surviving plants multiply rapidly and with this increase produce considerable slime possessing high absorptive properties. They break through the vessels and spread into the near-by parenchyma cells, with a gradual disintegration of this tissue resulting in the production of large bacterial pockets.

The bacteria travel up the stem, enter the vessels of the petiole, and mass to a great extent in the xylem elements of the pulvinus, this tissue being more succulent than the tissues of the hypocotyl, epicotyl, or petiole. From here the pathogene passes into the main vein of the leaf and thence into the smaller veins and veinlets. On the other hand, the bacteria may enter the stomata of the leaf, pass into the intercellular spaces of the parenchyma, enter the vessels, and thence pass into the vascular elements of the petiole and epicotyl.

From the xylem vessels of the stem the bacteria pass into those of the pedicel and peduncle and enter the sutures of the pod. From the dorsal suture they enter the funiculus (fig. 10, D), either disintegrating this structure to such an extent that the seeds fail to develop, or traveling through the raphe which leads into the seed coats, where the organism overwinters. The bacteria entering the pod cavity either from the funicular region or directly from the stomata of the pod make their way thence into the seed coats. The young plants may become infected at the time of germination, and the cycle is then repeated.

VARIETAL RESISTANCE

For a number of years past considerable progress has been made in the development of varieties of beans resistant to some of the common maladies. Most of this development, however, has dealt with bean anthracnose caused by *Colletotrichum lindemuthianum*, root rot caused by *Fusarium martii phaseoli*, and mosaic. Little has been accomplished in the breeding of varieties resistant to the common blight caused by *Bacterium phaseoli*.

The works of Burkholder (*6*) and Rands and Brotherton (*27*) on varietal resistance to bacterial blight are well known and need no review. Since these tests were carried on in New York and Michigan, respectively, where climatic conditions are somewhat different from those in Wisconsin, it was deemed advisable to duplicate these studies. The problem was carried on for a period of three summers, practically all of the commercial canning-bean varieties being used.

METHODS

Bacterial cultures for all inoculation experiments were grown in large quantities, which necessitated large surfaces upon which to

grow the organism. For this purpose liter flasks were at first employed, but it was later found that 500-c. c. culture bottles were more advantageous. About 1 inch of potato-dextrose agar was placed in the flask, sterilized, and then tipped upon its side to provide a larger surface. An ordinary test-tube culture of *Bacterium phaseoli* was washed off with sterile water, and the suspension poured into the flask containing the agar. These transfers were made under a hooded chamber, previously sterilized, to prevent contamination. The flasks containing the bacteria were allowed to incubate at 28° C. from three to five days, after which they were washed off with sterile water, and the suspension was placed in a hand spray pump.

The beans inoculated in the preliminary tests to establish a suitable method were grown in rows about 3 feet apart. Boxes were placed over the plants after they had been sprayed with the bacterial suspension, and a slight spray of water was directed over them for a period of 48 hours. The boxes were then removed and the plants examined daily for symptoms. Check plants were run in the same manner, but instead of the bacterial suspension distilled water was used. At the end of 12 days characteristic blight lesions were noticed on the inoculated plants, whereas the checks were free from blight.

The success of this method of inoculation led to a large-scale experiment in which 40 different varieties of beans were used, each variety consisting of one 40-foot row and the varietal rows 3 feet apart. A similar plot was planted as a check. With the use of a 3-gallon compressed-air sprayer the under side of the leaves could be covered easily with the inoculum. The plants were inoculated at various times of the day, and the amount of infection was noted in each case. Some were inoculated early in the morning, others in the late afternoon just before sunset, and still others early in the evening. The best results were noted on the plants that were inoculated in the late afternoon. The possible explanation for this is that the leaf stomata were open wider at that period than at other times of the day. Even though the sun was shining, it was not intense enough to cause any appreciable drying of the bacteria. Under these conditions a high percentage of infection resulted. When the plants were inoculated at night there was, without doubt, considerable moisture in the form of dew on the plants. The stomata, however, were not as wide open as during the day, as shown by greenhouse studies, and this probably accounted for the small amount of infection. The bacteria sprayed on the plants early in the morning probably dried to a large degree because of sunlight, and hence comparatively little infection resulted.

In order to produce a maximum amount of infection, the plants were inoculated at various stages of development, viz, before blossoming, during the blossoming period, and at the time of pod formation. Checkrows similar to those inoculated were allowed to grow normally. Observations on varietal resistance and susceptibility were made from time to time. A small amount of infection was noted in the check plots, having come about through natural spread from the inoculated plots.

During the summer of 1927 a third type of inoculation was carried on with fairly good results. The two varieties used for this experiment were the Refugee Wax, a fairly resistant variety, and Full

Measure, one quite susceptible. A very thick water suspension of the organism was made, and seeds of the two varieties mentioned were soaked in this suspension for about five minutes, after which they were planted. The suspension was then poured over the Refugee Wax seeds after being placed in the furrow, while the Full Measure seeds were covered with soil without the last treatment. The planting was made July 1. On July 27 these two varieties were examined for symptoms. The Refugee Wax showed very little infection, whereas the Full Measure was heavily infected. Besides having many leaf lesions, much infection was noted at the cotyledonary node, indicating that the bacteria, after the seed coats had been ruptured, had possibly made their way into the cotyledonary tissue and thence passed into the vascular elements of the epicotyl and hypocotyl, producing lesions at the point of entry into the seedlings. Even though both of these varieties were given the same soaking treatment, many more of the seed coats of the Refugee Wax variety than of the Full Measure were ruptured. This apparently had little effect on the amount of infection, since the Refugee Wax had fewer lesions as compared with the Full Measure, which showed a high degree of susceptibility. Since this type of inoculation is far more satisfactory than the ordinary type mentioned previously, because of the fact that weather conditions do not have to be taken into consideration for abundant infection, it is entirely possible that it would be feasible for use in varietal-resistance work.

VARIETAL TESTS

Table 2 shows the results of three summers' work on the problem of varietal resistance. All of the varieties listed were grown either in 1925, 1926, 1927, or in each of the three years. The amount of infection recorded as "very light" corresponds to any infection up to 5 per cent of the crop; "light" indicates approximately 25 per cent of the crop infected, "medium" about 50 per cent, "heavy" 65 per cent, "severe" about 80 per cent, and "very severe" that all of the plants were infected.

TABLE 2.—*Amount of infection on a number of commercial canning beans inoculated with Bacterium phaseoli in 1925, 1926, and 1927, under field conditions*

[±, Very light infection; +, light infection; ++, medium infection; +++, heavy infection; ++++, severe infection; +++++, very severe infection]

Average degree of infection	Type of bean	Variety	1925	1926	1927
ery light	Green	Rogers Stringless Green Refugee	±	±	±
		Refugee 1,000-1	±	±	±
		Extra Early Refugee	±		±
		Keeney Stringless Green Refugee	±	±	±
Light	do	Full Measure	++	+	±
		Low Champion	+		
		Burpee Fordhook Favorite bush bean		+	+
Do	Wax	Burpee New Kidney Wax	+	+	++
		Rogers Improved Kidney Wax		+	+
		Round Pod Kidney Wax	+	+	+
		Pencil Pod Wax	+	++	+
		Webber Wax		+	
		Rust proof Golden Wax			+
Medium	Green	Giant Stringless Green Pod	++	+	++
		Burpee Stringless Green Pod	++	++	+
		Dwarf Horticultural	+++	++	+++
		Longfellow	++	+	+++
		Improved Round Pod Valentine		+	+++

TABLE 2.—*Amount of infection, etc.*—Continued

Average degree of infection	Type of bean	Variety	1925	1926	1927
Medium	Wax	Sure Crop Wax	+	+++	++
		Olds Late Stringless Wax	----------	++	+
		Olds Early Stringless Wax	----------	±	+++
		Refugee Wax	++	++	++
		Hodson Wax	----------	++	+
Heavy	do	Improved Golden Wax	+++++	++++	+++
		Currie Rustproof Wax	----------	++++	+++
		Davis White Wax	----------	++	+++
		Burpee Black Wax	----------	+++	----------
Severe	Green	Bountiful	++++	++++	++++
		Black Valentine	----------	++++	++
		Wells Red Kidney	----------	++++	++++
Do	Wax	Old Style Wax	+++++	++++	+++
		Wardwell Wax	++++	++++	++++
Very severe	Green	Tennessee Green Pod	+++++	+++++	----------
Do	Wax	Crystal White Wax	----------	+++++	----------
		Keeney White-seeded Wax	----------	----------	+++++

From these results it can readily be seen that the different varieties of beans show considerable variation in their susceptibility to bacterial blight. There are no known varieties that show absolute resistance to the pathogene, but there are a few that exhibit a high degree of resistance. The Refugee types, comprising Extra Early Green Refugee, Refugee 1000–1, the Stringless Refugee, and Refugee Wax show little evidence of infection. Fortunately, most of these varieties are of an excellent type and quality and are used to a great extent by many of the Wisconsin canners. With the exception of the Refugee Wax, they are later than other varieties, and it has been suggested that possibly they owe their resistance to their lateness of maturity. Other varieties that show a medium degree of resistance coupled with good canning qualities are Giant Stringless Green Pod, Burpee Stringless Green Pod, Full Measure, Burpee New Kidney Wax, Round Pod Kidney Wax, and Rogers Improved Kidney Wax. Extremely susceptible varieties are Bountiful, Dwarf Horticultural, Tennessee Green Pod, Improved Golden Wax, Old Style Wax, Wardwell Wax, Currie Rustproof Wax, Crystal White Wax, Keeney White-seeded Wax, and Wells Red Kidney.

Table 2 shows that the results in each case over the 3-year period are not at all comparable. This can be explained because similar weather conditions did not prevail in the respective years which gave some variation in the degree of infection.

SUMMARY

Bacterial blight of beans caused by *Bacterium phaseoli* is seed borne and may cause characteristic lesions on stems, leaves, pods, and seeds. In cases of severe infection the seedling may often manifest a wilting, resulting from disintegration, toxic effects of the bacterial by-products, or plugging of the xylem vessels of the stem.

Infection is markedly influenced by moisture. Plants placed in a saturated humidity with the proper temperature and light show a high percentage of infection.

The bean-blight organism is widely distributed with infected seed. Local dissemination of the parasite may be brought about by dew.

rain, hail, wind, bean straw, insects, surface drainage, and irrigation waters. The importance of seed transmission is increased when the practice of inoculating the seed with a water suspension of the root-nodule organism, *Bacillus radicicola*, is employed, owing to the fact that this method of moistening the seed spreads the blight parasite from a few infected seeds over the entire lot.

Leaf infections are stomatal. Bacteria then invade the intercellular spaces, causing a gradual dissolution of the middle lamella. Later cell disintegration takes place, with the formation of bacterial pockets. Stem infection occurs through the stomata of the hypocotyl and epicotyl, through the vascular elements leading from the leaf to the stem, or from infected cotyledons. Bacteria in the xylem vessels may cause a wilting of the plant either by plugging of the vessels or by disintegration of the cell walls. Little infection is found in the secondary xylem because of the composition of the wall material.

Experimental evidence shows that the pathogene is harbored below the seed coats. The organisms pass into the sutures of the pods from the vascular system of the pedicel and make their way into the funiculus and thence through the raphe leading into the seed coats. Another method of entry into the seed is through the micropyle. No case of direct penetration through the seed coat has ever been observed.

The bacteria in the seed coats either remain there or pass into the region of the cotyledons and enter these structures when the seed germinates. Rifts in the epidermis of the cotyledon are formed after germination because of the increased size of the cells. Bacteria make their way through these tears, pass into the intercellular spaces of the cells below, and finally invade the entire cotyledon. Entrance might be made into the vascular elements whence infection of the young plant takes place.

Microchemical tests have shown that after germination much of the cotyledonary tissue becomes soluble, and bacterial action is probably influenced to a great extent through the solubility of material.

The data on varietal susceptibility to bacterial blight were collected under field conditions for three successive summers. No variety showed complete resistance; however, 4 varieties of the Refugee type showed a high degree of resistance, 19 showed medium resistance, and 12 showed little or no resistance.

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